

Award Number: W81XWH-10-2-0120

TITLE: Evaluation of the Human/Extreme Environment Interaction: Implications for Enhancing Operational Performance and Recovery

PRINCIPAL INVESTIGATOR: Brent C. Ruby, Ph.D., FACSM

CONTRACTING ORGANIZATION: University of Montana  
Missoula, MT 59812

REPORT DATE: October 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
1. REPORT DATE October 2011		2. REPORT TYPE Annual		3. DATES COVERED 1 October 2010 – 30 September 2011	
4. TITLE AND SUBTITLE  Evaluation of the Human/Extreme Environment Interaction: Implications for Enhancing Operational Performance and Recovery				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-2-0120	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Brent C. Ruby, Ph.D.  E-Mail: brent.ruby@mso.umt.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  University of Montana Missoula, MT 59812				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this research determines how hypoxia interacts with exercise and recovery to yield various metabolic responses that may affect performance and performance at high-altitude critical to mission success. Participants completed two maximal exercise tests under hypoxia, 3000 m and normoxic, 975 m conditions. Three experimental trials were then completed [hypoxia, 3000m at 60% of their hypoxic peak power (HH); normoxia, 975m at 60% of their hypoxic peak power (NH); and normoxia at 60% of their normoxic peak power (NN)] using a randomized, counterbalanced cross-over design over the span of 3 weeks, with a minimum of 7 days between trials. Our initial findings demonstrate that exercise during hypoxia (3000 m) and normoxia (975 m) results in similar responses (glycolytic, metabolic, and mitochondrial morphology genes, and oxidative stress markers) when the exercise intensity is clamped relative to the specific environment (as a % of either hypoxic or normoxic peak power). There were subtle differences in the glycogen response to exercise/recovery, which is likely a function of the higher absolute workrate during the NN trial. These results demonstrate similar response patterns across the three trials and suggest the need for a more aggressive degree of hypoxia to establish differences in the metabolic gene and oxidative stress marker responses.					
15. SUBJECT TERMS hypoxia, exercise recovery, oxidative stress, metabolic genes, mitochondrial genes					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	37	19b. TELEPHONE NUMBER (include area code)

**Table of Contents**

	<u>Page</u>
<b>Introduction.....</b>	<b>5</b>
<b>Body.....</b>	<b>6</b>
<b>Key Research Accomplishments.....</b>	<b>19</b>
<b>Reportable Outcomes.....</b>	<b>20</b>
<b>Conclusion.....</b>	<b>21</b>
<b>References.....</b>	<b>22</b>
<b>Appendices.....</b>	<b>28</b>

## **INTRODUCTION:**

Highly trained military forces must perform at peak performance during combat operations. The main goals of this proposal are to measure changes in muscle cells after exercise in cold and high altitude environments. Because the stresses of hard work can affect how well a soldier may perform on the next mission/assignment, we are also going to determine how nutrition can be used to improve recovery. This research plan uses methods that have direct application to the soldier's environment. The altitude stress included has direct relevance to the current conflicts and increased U.S. troop presence in Afghanistan. These studies have been developed to determine the effects of harsh environments on the body and what types of pre-mission training and what types of mission nutrition can improve performance, safety and recovery of the soldier. The application of these results should be used during early training of soldiers to increase the muscles adaptation(s) to training. During this initial year of the project series our specific aim was to determine the impact on metabolic genes and oxidative stress markers in response to exercising at intensities relative to hypoxic  $\text{VO}_2$  max in hypoxic conditions, relative to normoxic  $\text{VO}_2$  max in hypoxic conditions, and relative to normoxic  $\text{VO}_2$  max in normoxic conditions.



**BODY:**

The central scientific question from this research revolves around how hypoxia interacts with exercise and recovery to yield various metabolic adaptations that may affect performance and performance at high-altitude critical to mission success. The process of our work has been outlined below relative to our original statement of work. At present we are slightly ahead of schedule due to earlier than anticipated IRB/HRPO approval of our methodologies for the second laboratory study.

**Year 1****Task 1. Submission of IRB protocols for human subjects testing (months 1-6).**

This has been completed for the initial protocol. Additional details regarding the additional protocol specifics are outlined below.

- 1a.** Submission of University approved IRB for laboratory study 1 to Army for review (month 1).

The University IRB approved protocol for laboratory study 1 was submitted to the Army mid-January for review and approval.

- 1b.** Submission of IRB applications for laboratory studies 2 and 3, and field study 1 to university IRB for review and approval (month 1).

We are requesting an alteration of this task to better reflect the goals of studies 2, 3 and the field trial. The rationale for this request relates to the concept that the subsequent studies build on the initial data collection and results. We want to be able to incorporate necessary changes to either the environmental conditions and/or the exercise protocols based on findings from study 1. This is currently underway as we complete the analyses and these adjustments have been incorporated into the next IRB submission (laboratory study 2). Study 2 has just recently been approved by our University IRB and by HRPO.

- 1c.** Receive University IRB approval for laboratory studies 2 and 3, and field study 1 and submit to Army for review and approval (months 1-6).

See above 1a.

- 1d.** Receive Army approval for all protocols (months 2-6).

Approval for the initial study was obtained as indicated above. Approval for the second laboratory study has recently been reviewed and approved in September.

**Task 2. Preparation of laboratory for data collection (months 1-6).**

- 2a.** Place orders for required equipment purchases, University bid process (months 1-2).
- 2b.** Receive equipment and set up for experimental protocols (months 2-4).

These tasks have all been completed and we are on schedule as noted in our original statement of work.

**Task 3. Data collection for laboratory study 1 (months 6-9).**

**3a.** Finalize subject recruitment and initiate testing (months 6-7).

**3b.** Data collection (months 6-9).

This has been completed.

**Task 4. Data analyses for laboratory study 1 (months 9-14).**

This has been nearly completed. All blood and muscle samples have been analyzed as planned and we are on schedule.

**4a.** Order necessary analytical kits, probes, primers and other analyses needs (month 9).

This has been completed.

**4b.** Finalize analytical techniques and analyze all samples (months 9-12).

This has been completed.

**4c.** Data analyses of descriptive and other data collected (non biochemical) (months 9-10).

This has been completed.

**4d.** Statistical analyses of all data from study 1 (months 13-14).

This has been completed and the initial two research manuscripts are nearly complete. We are ahead of schedule for this task because it has been critical to assess findings from year one to adapt the methodologies accordingly for the second laboratory study. All of the findings were discussed during a meeting with all researchers present in August, 2011. From these findings, we determined that the degree of hypoxia needed to be increased. This approach was built into the methodology of the proposal and has provided us the flexibility to adjust the chamber conditions based on initial findings. These are further discussed in the annual report.

This task has been completed during the initial year of the project ahead of schedule.

**Task 5. Data collection for lab study 2 (months 12-16).**

**5a.** Revise approved IRB protocols for any necessary changes suggested by laboratory study 1 and recruit subjects (months 12-13).

Based on our discussions regarding minor adjustments to the experimental conditions, we completed the second IRB application, which was reviewed in late August, 2011. This

application was completed immediately after our research team discussions and was approved by the University in late August 2011. Following University IRB approval, the application was submitted on August 31 by our Center to HRPO at Fort Detrick. Because of our initial work with Sharon Evans on our first IRB submission (lab study 1), all our paperwork was compiled and submitted quickly. HRPO approval was granted on September 22, 2011 (a new record for our research Center as we typically anticipate a 2-3 month review and approval process).

This has put us ahead of schedule and has allowed us to coordinate some maintenance to our chamber and to better prepare us for an early start to the data collection period for the next laboratory study. We will be initiating pilot testing for this phase of the project by mid-November.

Data collection for study one was completed with the intention of determining the interactions between muscle work under hypoxic conditions and recovery on the metabolic responses and/or early cell signaling and metabolic adaptations. However, additional findings relative to the oxidative stress response to the exercise and recovery periods have also been noted. These findings are discussed in more detail below and are being prepared as separate manuscripts.

## **Findings from year 1**

### **Introduction**

It has been largely assumed since 1962 that hypoxic conditions and/or altitude stimulate muscle oxidative capacity. This notion comes from the observation that active Peruvian miners had 78% more cytochrome c reductase and 16% more myoglobin than low land controls (56). Five years later it was noted that endurance exercise was a potent stimulator of mitochondrial enzymes (29). The data from these two studies formed the consensus that muscle hypoxia (from exercise or environment) was an important stimulus for mitochondrial development. In the early 1990's this tenant was challenged by data before and after mountaineer expeditions to the Himalayas. The findings of these studies demonstrated a loss of muscle cross sectional area, decreased mitochondrial volume, and decreased maximal aerobic capacity (20,31,32). Additionally, after return from these expeditions lipofuscin, a mitochondrial breakdown by-product, was increased (44). These findings of reduced oxidative capacity were further confirmed by "Expedition Everest II" (42). Low levels of mitochondrial capacity have also been shown in the high altitude residents of La Paz (18) and in Sherpa's (37). Paradoxically, these high land populations have excellent physical performance at altitude.

When exercise training under hypoxic conditions, but recovering in a normoxic environment, a slightly different story emerges (train high, live low). When this type of short-term exposure is incorporated into a training paradigm mitochondrial density, maximum aerobic capacity, citrate synthase activity, and anaerobic performance is enhanced over normoxic control exercise (5,17,23,27,46,47,63,64). Functionally, increased glucose metabolism shown with altitude exposure and acclimatization (12) may be more efficient in that this adaptation would lead to more ATP per molecule of oxygen. The paradox with short-term and long-term

metabolic adaptations is yet to be completely understood, however it may be related to the recovery process and specifically indications of decreased protein synthesis during hypoxia (26).

The mechanisms to which hypoxic adaptations occur are even less clear, but seem to be regulated by hypoxia-inducible factor 1 (HIF-1) (59). HIF-1 is a transcription factor that stabilizes in the nucleus upon exposure to hypoxic conditions (35,62) and in turn induces the expression of hypoxia-induced genes. HIF-1 causes increased gene expression for glucose transporters, glycolytic enzymes, angiogenic factor, and erythropoietin by targeting the hypoxic response element in the promoter region (52,59). Higher HIF-1 mRNA levels after training (64,69) may produce an increased potential for quick activation of HIF-1 with the onset of hypoxic stimuli after training at altitude (30). However, the response of HIF-1 after exercise training is blunted in response to an absolute intensity exercise protocol (41). Thus, HIF-1 stabilization may be increased by exercise and factors independent of hypoxia. Indeed, interleukin-1 $\beta$ , insulin like growth factor I and II, insulin, heregulin, epidermal growth factor TNF  $\alpha$ , angiotensin-2, and nitric oxide have been shown to be capable of inducing HIF-1 (19,25,28,39,57,68). The complex nature of HIF-1 activation serves a common endpoint to delivery oxygen and up-regulate the metabolic machinery of the cell.

Peroxisome proliferator-activated receptor gamma coactivator 1  $\alpha$  (PGC-1 $\alpha$ ) gene expression is strongly induced by exercise (2,38,50) contributing to mitochondrial biogenesis and metabolic alterations. Recent data from our lab has shown PGC-1 $\alpha$  mRNA to increase with acute exercise, but have a blunted response after 11 and 21 days of endurance training (Figure 1). PGC-1 $\alpha$  expression is also induced by hypoxia (1) and does not appear to be dependent on HIF-1. The activation of PGC-1 $\alpha$  stimulates mitochondrial biogenesis and hence contributes to increased fatty acid fuel oxidation and subsequent exercise performance (40,66).

The current view of the mitochondria is not one of many mitochondria, but of a single mitochondrion within a cell where the solitary shapes of mitochondria are considered to be sections of mitochondrial tubules. Fusion (growth and development) of these tubules has been shown in healthy conditions such as exercise (6) and weight loss (22,48) while fission (degradation, dysfunction) is associated with obesity and disease (3,4). We have recently observed an increase in Mitofusin 2 (MFN2) mRNA a mitochondrial fusion gene with acute exercise throughout 21 days of endurance training. It has been suggested that the balance between fusion and fission may play a central role in the metabolic consequences of hypoxia (36).

These two distinct pathways of metabolic alteration with hypoxia (1. HIF-1 and 2. PGC-1 $\alpha$ ) have very different effects. The HIF-1 pathway leads to increased reliance on carbohydrate substrates and the PGC-1 $\alpha$  pathway leads to increased reliance on fat substrates. Clearly, investigations are needed to discern the pathways and applied metabolic outcomes associated with hypoxia. This information will allow for protocol development for not only altitude/hypoxia tolerance, but also for exercise performance, both of which may be critical to mission success in harsh environments.

Exercise intensities during exercise studies are usually determined by incorporating intensity relative to maximal aerobic capacity (i.e. 60% VO<sub>2</sub> max). This becomes a challenge when studying the effects of hypoxia and altitude versus normoxic control conditions because acute hypoxia decreases VO<sub>2</sub> max when compared to normoxic conditions (16,21,24,43,53,58,61,67). Endurance trained individuals with an average sea level VO<sub>2</sub> max of 65.5 ml/kg/min will have an average VO<sub>2</sub> max at 2500 m of 57.7 ml/kg/min, a 7.8% decrease (49). When exercise intensity is set at 65% of environmental VO<sub>2</sub> max (as is typically done) the absolute exercise intensities will differ in that the hypoxic intensity will be at a lower absolute intensity (42.58 ml/kg/min under normoxic conditions and 37.51 ml/kg/min under hypoxic conditions). Thus difference in absolute intensity may be a contributing factor (other than hypoxia) to studies incorporating this method of choosing exercise intensity. Therefore, the purpose of this project was to determine the impact on metabolic genes in response to exercising at an intensity relative to hypoxic VO<sub>2</sub> max in hypoxic conditions, relative to normoxic VO<sub>2</sub> max in hypoxic conditions, and relative to normoxic VO<sub>2</sub> max in normoxic conditions.

## **METHODS**

### ***Participants***

Twelve male participants (24 ± 1 yrs, 183 ± 2 cm, 82 ± 3 kg, 4.2 ± 0.3 L•min<sup>-1</sup> (975 m), 4.0 ± 0.2 L•min<sup>-1</sup> (3000 m)) completed the study. Subjects completed a Physical Activity Readiness Questionnaire (PAR-Q) and were briefed on the experimental protocol and possible risks prior to giving written informed consent. All procedures were approved by the University Institutional Review Board (The University of Montana, Missoula, MT).

### ***Preliminary Testing***

Body composition was measured using hydrodensitometry. Underwater mass was measured with a digital scale (Exertech, Dreshbach, MN). Body Density was corrected for estimated residual lung volume (10) and converted to percent body fat using the Siri equation (60). Two graded maximal exercise tests (starting at 95 W, and increasing 35 W every 3 minutes) were completed (minimum 2 days between tests) on an electronically braked cycle ergometer (Velotron, RacerMate Inc., Seattle, WA) to determine maximal aerobic capacity (VO<sub>2max</sub>) and the power output associated with VO<sub>2max</sub> (W<sub>max</sub>) at 975 m and 3000 m. Expired gases were collected and analyzed during the test, using a calibrated metabolic cart (ParvoMedics, Inc., Salt Lake City, UT). VO<sub>2max</sub> was assigned to the highest achieved oxygen uptake recorded during the test. W<sub>max</sub> was calculated by adding the Watts in the last completed stage to the fraction of time spent in the uncompleted stage multiplied by 35.

### ***Experimental Protocol***

*Design.* Participants completed 3 trials [one exercise trial in the simulated altitude environment at an intensity of 60% of their hypoxic peak power (HH); one exercise trial in the normal lab altitude at an intensity of 60% of their hypoxic peak power (NH); and one exercise trial in the normal lab altitude at an intensity of 60% of their normoxic peak power (NN)] using a randomized, counterbalanced cross-over design over the span of 3 weeks, with a minimum of 7 days between trials. All trials were completed in a temperature, humidity, and hypoxia (Colorado Altitude Training, Louisville, CO) controlled environmental chamber (Tescor,

Warminster, PA) in ambient conditions of 12°C and 40% relative humidity. Participants kept an exercise record for 2 days before and a dietary record for 24 h before the initial trial and replicated exercise and diet for these periods before the remaining trials. Additionally, participants abstained from exercise 24 hours before each trial. Following an overnight 12 hour fast, participants arrived at the laboratory in the early morning to complete testing. Upon arrival to the laboratory nude body mass was measured (CW-11, Ohaus Corporation, Pine Brook, NJ). The trials consisted of 1) cycling for 1 hour at 60% of peak power (as measured at 975 m) at 975 m and recovering at 975 m for 4 hours, 2) cycling for 1 hour at 60% of peak power (as measured at 3000 m) at 975 m and recovering at 975 m for 4 hours, and 3) cycling for 1 hour at 60% of peak power (as measured at 3000 m) at 3000 m and recovering at 3000 m for 4 hours. During the recovery period, participants changed out of their cycling clothes, towed off, and wore a standardized sweat suit to mitigate shivering. Participants remained in a sitting position throughout the 4 hour recovery period. Nude body mass was measured following the ride and at the end of the 4 hour recovery period. Participants consumed 8 ml•kg•min<sup>-1</sup> during the ride and 8 ml•kg•min<sup>-1</sup> during the recovery.

*Biopsies.* Muscle biopsies were taken before exercise and at the end of the 4 hour recovery period for each trial. Biopsies were taken from the *vastus lateralis* muscle using a 4-5 mm Bergstrom percutaneous muscle biopsy needle with the aid of suction (8). All subsequent biopsies during a given trial were obtained from the same leg using a separate incision 2 cm proximal to the previous biopsy. After excess blood, connective tissue, and fat were removed, tissue samples were stored in RNA Later or immersed in liquid nitrogen and stored at -80°C for later analysis.

*Blood Samples.* A 10 mL and 6 mL blood sample was taken from an antecubital vein before exercise and at 0, 2, and 4 hours during recovery. Immediately, 2 capillary tubes were filled with 100ul of blood each and placed in a hematocrit centrifuge for 3 minutes, 15,290 x g (A13, Jouan, Winchester, VA) for analysis of hematocrit. The proportion of blood cells to serum was measured in each tube and the average of the two measures was used to determine hematocrit. The remainder of the blood sample was kept at room temperature for 20 minutes to allow clotting. Once clotted the samples were centrifuged at 7500 x g for 20 minutes (MR 22i, Jouan, Winchester, VA). Serum was removed and stored at -80°C until subsequent analyses.

*Respiratory Parameters.* Expired gases were collected during exercise at 0, 27.5, and 53 min of exercise to evaluate differences in oxygen consumption and respiratory function among trials. Each collection was collected for 5 min, with the last 2 min averaged to represent the sample period.

*Pulse Oximetry.* Blood oxygen saturation was evaluated pre ride, during the ride at 4, 31.5, and 57 mins, and at 0, 2, and 4 hours during the recovery using a pulse oximeter (Nonin Onyx II 9550, Plymouth, MN).

## ***Analysis***

***Skeletal Muscle RNA isolation.*** An 8-20 mg piece of skeletal muscle will be homogenized in 800ul of Trizol using an electric homogenizer. The samples are then incubated at room temperature for 5 minutes after which 200ul of chloroform per 1000ul of Trizol is added and shaken vigorously by hand. After an additional incubation at room temperature for 2-3 minutes the samples are centrifuged at 12,000 g for 15 minutes and the aqueous phase was transferred to a fresh tube. The RNA is then precipitated by adding 500ul of isopropyl alcohol pre 1000ul of initial Trizol and incubated overnight at -20°C. The next morning samples are centrifuged at 12,000g for 10 minutes at 4°C and the RNA is washed by removing the supernatant and adding 1000ul of 75% ethanol per 1000ul of initial Trizol. The samples are then mixed by vortex and centrifuged at 7,500g for 5 minutes at 4°C. The RNA is then redissolved in 100 ul RNase-free water after the supernatant is removed and the RNA pellet was dried. The RNA is then cleaned using the RNeasy mini kit (Qiagen) according to the manufactures protocol using the additional DNase digestion step (DNA mini kit, Qiagen). RNA is then quantified using a nano-spectrophotometer.

***cDNA synthesis.*** First-strand cDNA synthesis is achieved using Superscript-first strand kit (Invitrogen) according to the manufactures protocol. Each sample within a given subject will contain the same amount of RNA (400 – 1000 ng). The resulting cDNA was then diluted using RNase free water in order to have adequate volume for RT-PCR and frozen for later RT-PCR analysis.

***Real time RT-PCR.*** Each 25ul reaction volume will contain 500nM primers, 250nM probe (PrimeTime qPCR assay, Integrated DNA technologies), 1x FastStart TaqMan Probe master (Roche Applied Science), and 2.5 ul of sample cDNA. PCR will then run using the Bio-Rad iCycler iQ5 Real-Time PCR Detection system (Bio-Rad) using a 2-step Roche protocol.

***Oxidative Stress.*** Plasma aliquots were assayed for an oxidative stress biomarker panel used previously by our group (33,55). Two biomarkers, plasma trolox-equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant potential (FRAP), were chosen to evaluate blood plasma antioxidant capacity. Oxidative damage was evaluated in plasma by determination of protein carbonyls and lipid hydroperoxides content.

***Antioxidant Capacity.*** Plasma antioxidant capacity was measured by the plasma trolox-equivalent antioxidant capacity technique whereby a radical cation of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) reaction is quenched by sample-specific antioxidant fortifications (15). Total plasma antioxidant potential was determined by the ferric reducing antioxidant potential assay according to the methodology of (7). Both TEAC and FRAP assays result in quantifiable colorimetric solutions which are visualized spectrophotometrically. Lipid peroxidation was determined by the ferrous oxidation-xylenol orange assay (51). In brief, ferrous ions are oxidized by lipid hydroperoxides to ferric ions and subsequently react with the ferrous sensitive dye containing xylenol orange. In the presence of lipid hydroperoxides, this reaction forms a spectrophotometrically quantifiable complex. Protein carbonyls were analyzed using a commercially available ELISA kit (Zentech Technology, Dunedin, New Zealand). All assays were performed in triplicate and exhibited

within sample coefficients of variation between 2% and 5%. Prior to analysis, all plasma samples were assayed in quadruplicate for protein concentration based on the methods of Bradford et al, (1976) and adjusted to 4 mg/ml protein using a phosphate buffer (11). All oxidative stress biomarkers were normalized for plasma volume shifts experienced during the three trials.

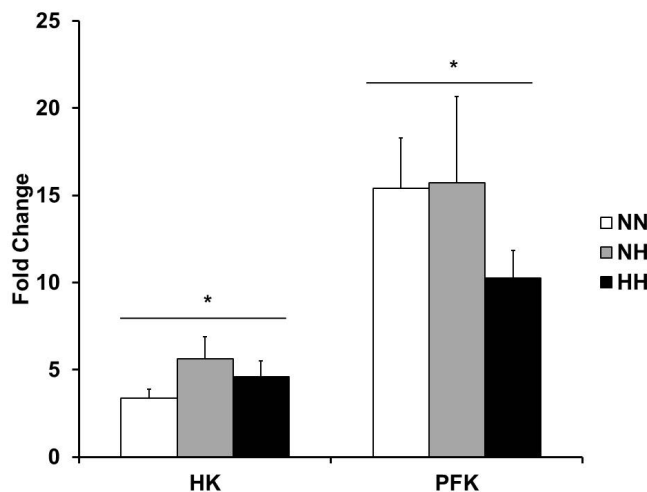
## Statistics

Muscle glycogen, substrate utilization, mRNA of metabolic genes and oxidative stress markers were analyzed using a repeated measure ANOVA (trial\*time). A probability of type I error less than 5% was considered significant ( $p < 0.05$ ). All data is reported as means  $\pm$  SE.

## RESULTS:

### Glycolysis Genes:

HK and PFK increased as a result of the exercise and recovery ( $p < 0.001$  and  $p < 0.001$  respectively) but were not different between trials ( $p = 0.126$  and  $p = 0.419$  for HK and PFK, respectively).

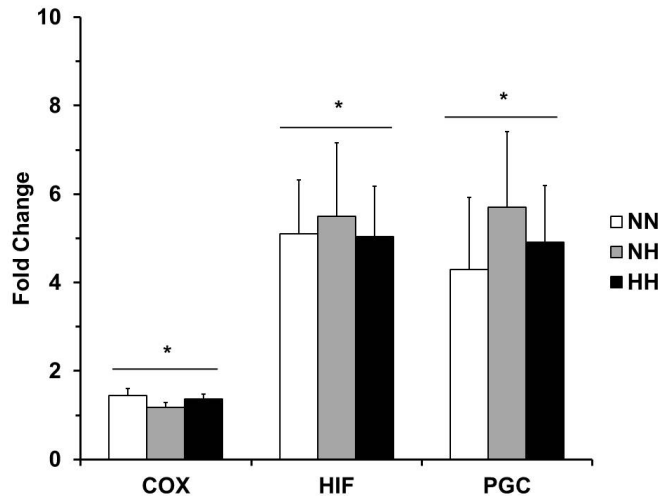


**Figure 1.** Changes in HK and PFK in response to the three exercise/recovery conditions. \* $p < 0.05$ , main effect of time. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

### Metabolic Genes:

COX, HIF, and PGC increased as a result of the exercise and recovery ( $p < 0.001$ ,  $p = 0.002$ , and  $p = 0.003$  respectively) but were not different between trials ( $p = 0.342$ ,  $p = 0.951$ , and  $p = 0.849$  for COX, HIF, and PGC, respectively).

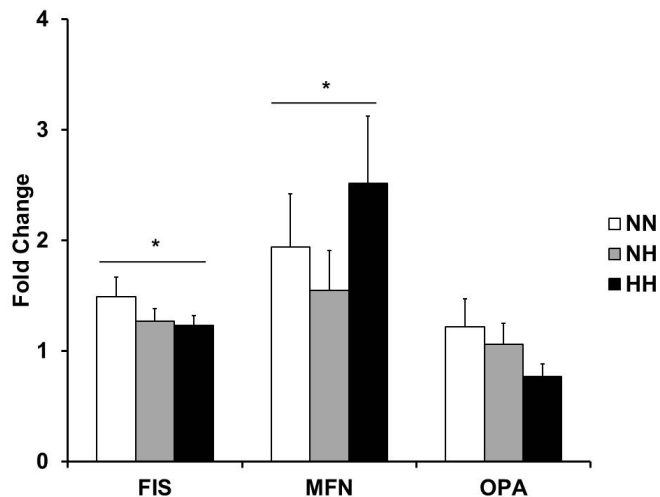




**Figure 2.** Change in COX, HIF, and PGC in response to the three exercise/recovery conditions. \* $p < 0.05$ , main effect of time. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

#### *Mitochondrial Morphology Genes:*

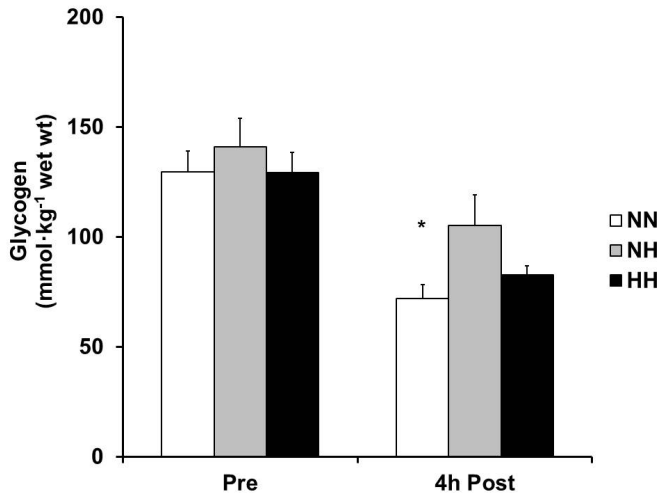
FIS, and MFN increased as a result of the exercise and recovery ( $p < 0.007$  and  $p = 0.001$  respectively) but OPA did not ( $p = 0.923$ ). FIS, MFN, and OPA were not different between trials ( $p = 0.368$ ,  $p = 0.374$ , and  $p = 0.068$ , respectively).



**Figure 3.** Changes in FIS, MFN, and OPA (\* $p < 0.05$  vs. main effect for time) in response to the three exercise/recovery conditions. \* $p < 0.05$ , main effect of time. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

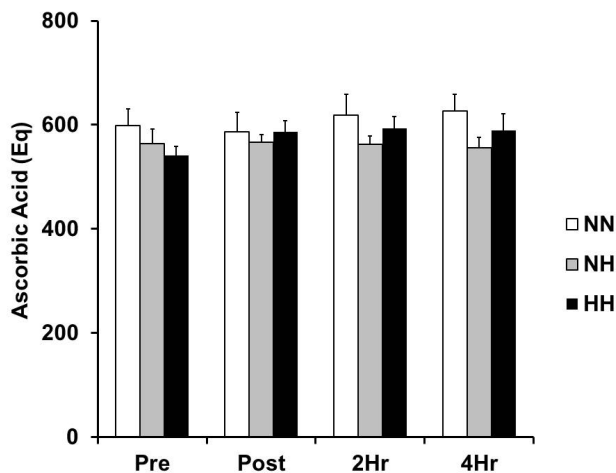
### Glycogen:

Muscle glycogen was similar pre exercise between all three trials ( $p>0.05$ ). Muscle glycogen was lower at 4 h post in the NN trial vs. the NH trial ( $p=0.030$ ), but was not different among other trials ( $p>0.05$ ).



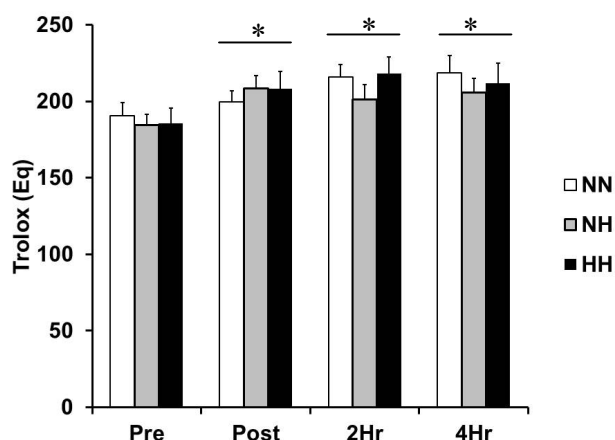
**Figure 4.** Changes in muscle glycogen in response to the three exercise/recovery conditions. \* $p<0.05$ , interaction effect. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

*Ferric reducing ability of plasma (FRAP):* Trial main effects were noted for FRAP (Trial  $p=0.0138$ , Time  $p=0.241$ ), and cannot be explained currently.



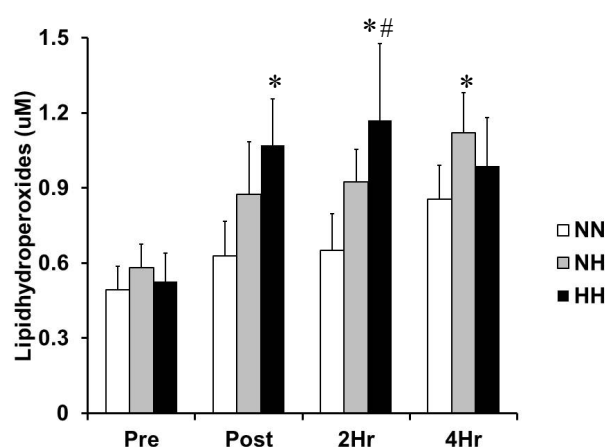
**Figure 5.** Changes in ferric reducing ability of plasma (FRAP) in response to the three exercise/recovery conditions. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

*Trolox equivalent antioxidant capacity of plasma (TEAC):* Time main effects for TEAC (Trial  $p = 0.263$ , Time  $p < 0.0001$  (Pre vs. Post, 2Hr, & 4Hr) likely reflect an increase in circulating plasma urate due to the 3 exercise bouts.



**Figure 6.** Changes in trolox equivalent antioxidant capacity of plasma (TEAC) in response to the three exercise/recovery conditions. \* $p < 0.05$ , main effect of time. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

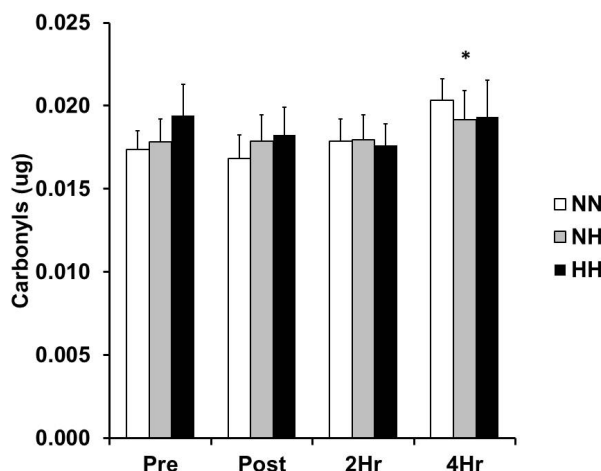
*Plasma lipid hydroperoxide (LOOH):* LOOHs exhibited the most dramatic response to the 3 exercise trials (Main effects: Trial  $p = 0.009$ , Time  $p < 0.0001$ ) with significant increases being observed following both the high altitude simulation trials. Trial HH elicited an increase in LOOH Post and 2Hr time points, while Trial NN and HH were different at 2Hr. Moreover, NH LOOH values were increased over at 4Hr post exercise as compared to Pre.



**Figure 7.** Changes in plasma lipid hydroperoxide (LOOH) in response to the three exercise/recovery conditions. \*Different from Pre, main effect of time,  $p < 0.05$ . #Different from

NN, main effect trial,  $p < 0.05$ . (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

*Plasma protein carbonyls (PC):* A similar Time main effect was noted for PCs, indicating a rise in circulating oxidized proteins following exercise, though no trial main effects were noted (Main effects: Trial  $p = 0.577$ , Time  $p = 0.012$  for 4Hr vs. Post and 2Hr time points).



**Figure 8.** Changes in plasma protein carbonyls (PC) in response to the three exercise/recovery conditions. \* $p < 0.05$ , main effect of time vs. Post and 2Hr time points. (NN = normal lab altitude at an intensity of 60% of their normoxic peak power; NH = normal lab altitude at an intensity of 60% of their hypoxic peak power; HH = simulated altitude environment at an intensity of 60% of their hypoxic peak power)

## SUMMARY

### *Metabolic Genes*

The main finding here is the lack of difference between trials for our select metabolic genes. This is an important finding in that it allows us to optimize future protocols to distinguish a gradient of adaptation between trials. Specifically, we suggest increasing the amount of hypoxia in the simulated high altitude trials. These subtle alterations in the hypoxic environment have been included in the second year methodology and have received IRB/HRPO approval. These methodologies can be reviewed in more detail in Appendix 1.

### *Oxidative stress markers*

For the current experiments we examined four key biomarkers of blood plasma oxidative stress, ferric reducing ability of plasma (FRAP), trolox equivalent antioxidant capacity of plasma (TEAC), lipid hydroperoxide (LOOH), and protein carbonyls (PC). These biomarkers were chosen for their sensitivity to exercise (9,33,34,45,54,55) in addition to the biological pecking order for oxidative stress reactions in complex biological fluids such as blood plasma

(13,14,65). This biomarker panel is sensitive to aqueous and lipid phase antioxidants. The TEAC and FRAP assays are uniquely sensitive for urate and vitamin C. These antioxidants account for upwards of 70% of the antioxidant capacity of plasma. Moreover, this biomarker panel is sensitive to protein and non-protein thiol antioxidant capacity.

Data from this study reveal only modest changes in our plasma oxidative stress marker panel. There was a significant main effect for time for TEAC, which likely reflects an increase in circulating plasma urate in response to each of the exercise bouts. A similar main effect for time was noted for PCs, indicating a rise in circulating oxidized proteins following exercise, though no trial main effects were noted. Trial main effects were noted for FRAP, an observation that cannot be explained currently. LOOHs exhibited the most dramatic response to the exercise trials with significant increases being observed following both the hypoxic trials.

**KEY RESEARCH ACCOMPLISHMENTS:**

- Statement of work tasks 1-4 are complete.
- Task 4d has been completed ahead of schedule.
- The phase 2 laboratory study has been revised based on initial findings from year 1 efforts and has received University IRB and HRPO approval.
- A manuscript focusing on the oxidative stress findings from study 1 is being prepared.
- A manuscript focusing on the mitochondrial gene response findings from study 1 is being prepared.

**REPORTABLE OUTCOMES:**

- A manuscript focusing on the oxidative stress findings from study 1 is being prepared.
- A manuscript focusing on the mitochondrial gene response findings from study 1 is being prepared.

## CONCLUSIONS:

The purpose of this research determines how hypoxia interacts with exercise and recovery to yield various metabolic responses that may affect performance and performance at high-altitude critical to mission success. In the present study design, participants completed two maximal exercise tests under hypoxia, 3000 m and normoxic, 975 m conditions to establish experimental work rates. Three experimental trials were then completed [hypoxia, 3000m at 60% of their hypoxic peak power (HH); normoxic, 975m at 60% of their hypoxic peak power (NH); and normoxia at 60% of their normoxic peak power (NN)] using a randomized, counterbalanced, cross over design over the span of 3 weeks, with a minimum of 7 days between trials.

Our initial findings demonstrate that exercise in hypoxic (3000 m) and normoxic (975 m) environments results in similar responses (glycolytic, metabolic, and mitochondrial morphology genes, and oxidative stress markers) when the exercise intensity is clamped relative to the specific environment (as a % of either hypoxic or normoxic peak power). Although there were subtle differences in the glycogen response to exercise/recovery, this is likely a function of the higher absolute work rate during the NN trial.

These results demonstrate similar response patterns across the three trials and suggest the need for a more aggressive degree of hypoxia to establish differences in the metabolic gene and oxidative stress marker responses. Based on these initial findings, we have altered the hypoxic environment for the phase II recovery study. The two environmental stresses we will be utilizing during recovery include normoxic (975 m) and a more aggressive hypoxic stress (5000 m). This is further detailed in the attached appendices (IRB and HRPO protocol approvals).

From a practical standpoint, the present data demonstrate that acute exercise stress under normoxic or hypoxic conditions results in similar physiological responses that may set the stage for subsequent adaptations. That is, the degree of metabolic and mitochondrial gene activation is similar between the hypoxic and normoxic environments in response to the acute exercise bout and recovery period. This is also true for the response of several gold standard oxidative stress markers. Therefore, the exercise intensities subjects may self-select under varied degrees of environmental stress (hypoxia), although different, result in similar degrees of physiological stress and potential for metabolic adaptation.

These results demonstrate thus far that the responses of metabolic and mitochondrial genes and markers of oxidative stress are driven by exercise intensity and that potential for metabolic adaptations are similar across environments. Therefore, the expected training responses and cellular remodeling appear to respond similarly as a function of the relative exercise intensities. This may further suggest that intensities participants self-select during acute exercise in hypoxic and normoxic environments have the potential to demonstrate similar metabolic adaptations despite variations in the absolute work rates.



## REFERENCES:

1. Arany, Z, Foo, SY, Ma, Y, Ruas, JL, Bommi-Reddy, A, Girnun, G, Cooper, M, Laznik, D, Chinsomboon, J, Rangwala, SM, Baek, KH, Rosenzweig, A, Spiegelman, BM. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* 451: 1008-1012, 2008.
2. Baar, K, Wende, AR, Jones, TE, Marison, M, Nolte, LA, Chen, M, Kelly, DP, Holloszy, JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 16: 1879-1886, 2002.
3. Bach, D, Naon, D, Pich, S, Soriano, FX, Vega, N, Rieusset, J, Laville, M, Guillet, C, Boirie, Y, Wallberg-Henriksson, H, Manco, M, Calvani, M, Castagneto, M, Palacin, M, Mingrone, G, Zierath, JR, Vidal, H, Zorzano, A. Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. *Diabetes* 54: 2685-2693, 2005.
4. Bach, D, Pich, S, Soriano, FX, Vega, N, Baumgartner, B, Oriola, J, Dagaard, JR, Lloberas, J, Camps, M, Zierath, JR, Rabasa-Lhoret, R, Wallberg-Henriksson, H, Laville, M, Palacin, M, Vidal, H, Rivera, F, Brand, M, Zorzano, A. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J Biol Chem* 278: 17190-17197, 2003.
5. Bailey, DM, Davies, B, Baker, J. Training in hypoxia: modulation of metabolic and cardiovascular risk factors in men. *Med Sci Sports Exerc* 32: 1058-1066, 2000.
6. Bailey, DM, Young, IS, McEneny, J, Lawrenson, L, Kim, J, Barden, J, Richardson, RS. Regulation of free radical outflow from an isolated muscle bed in exercising humans. *Am J Physiol Heart Circ Physiol* 287: H1689-1699, 2004.
7. Benzie, IF, Strain, JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 239: 70-76, 1996.
8. Bergstrom, J. Muscle electrolytes in man. *Scand J Clin Lab Invest* 14: 7-110, 1962.
9. Bloomer, RJ, Ferebee, DE, Fisher-Wellman, KH, Quindry, JC, Schilling, BK. Postprandial Oxidative Stress: Influence of Sex and Exercise Training Status. *Med Sci Sports Exerc* 2009.
10. Boren, H, Kory, RC, Syner, JC. The Veteran's Administration-Army cooperative study of pulmonary function: II. The lung volume and its subdivisions in normal men. *Am J Med* 41: 96-114, 1966.
11. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254, 1976.

12. Brooks, GA, Butterfield, GE, Wolfe, RR, Groves, BM, Mazzeo, RS, Sutton, JR, Wolfel, EE, Reeves, JT. Increased dependence on blood glucose after acclimatization to 4,300 m. *J Appl Physiol* 70: 919-927, 1991.
13. Buettner, GR. the pecking order of free radicals and antioxidants: -tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics* 300: 535-543, 1993.
14. Cao, G, Prior, RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* 44: 1309-1315, 1998.
15. Cao, G, Russell, RM, Lischner, N, Prior, RL. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J Nutr* 128: 2383-2390, 1998.
16. Chapman, RF, Emery, M, Stager, JM. Degree of arterial desaturation in normoxia influences VO<sub>2</sub>max decline in mild hypoxia. *Med Sci Sports Exerc* 31: 658-663, 1999.
17. Desplanches, D, Hoppeler, H, Linossier, MT, Denis, C, Claassen, H, Dormois, D, Lacour, JR, Geyssant, A. Effects of training in normoxia and normobaric hypoxia on human muscle ultrastructure. *Pflugers Arch* 425: 263-267, 1993.
18. Favier, R, Spielvogel, H, Desplanches, D, Ferretti, G, Kayser, B, Hoppeler, H. Maximal exercise performance in chronic hypoxia and acute normoxia in high-altitude natives. *J Appl Physiol* 78: 1868-1874, 1995.
19. Feldser, D, Agani, F, Iyer, NV, Pak, B, Ferreira, G, Semenza, GL. Reciprocal positive regulation of hypoxia-inducible factor 1 $\alpha$  and insulin-like growth factor 2. *Cancer Res* 59: 3915-3918, 1999.
20. Ferretti, G, Boutellier, U, Pendergast, DR, Moia, C, Minetti, AE, Howald, H, di Prampero, PE. Oxygen transport system before and after exposure to chronic hypoxia. *Int J Sports Med* 11 Suppl 1: S15-20, 1990.
21. Ferretti, G, Moia, C, Thomet, JM, Kayser, B. The decrease of maximal oxygen consumption during hypoxia in man: a mirror image of the oxygen equilibrium curve. *J Physiol* 498 ( Pt 1): 231-237, 1997.
22. Gastaldi, G, Russell, A, Golay, A, Giacobino, JP, Habicht, F, Barthassat, V, Muzzin, P, Bobbioni-Harsch, E. Upregulation of peroxisome proliferator-activated receptor gamma coactivator gene (PGC1A) during weight loss is related to insulin sensitivity but not to energy expenditure. *Diabetologia* 50: 2348-2355, 2007.
23. Geiser, J, Vogt, M, Billeter, R, Zuleger, C, Belforti, F, Hoppeler, H. Training high--living low: changes of aerobic performance and muscle structure with training at simulated altitude. *Int J Sports Med* 22: 579-585, 2001.

24. Gore, CJ, Hahn, AG, Scroop, GC, Watson, DB, Norton, KI, Wood, RJ, Campbell, DP, Emonson, DL. Increased arterial desaturation in trained cyclists during maximal exercise at 580 m altitude. *J Appl Physiol* 80: 2204-2210, 1996.
25. Gorlach, A, Diebold, I, Schini-Kerth, VB, Berchner-Pfannschmidt, U, Roth, U, Brandes, RP, Kietzmann, T, Busse, R. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: Role of the p22(phox)-containing NADPH oxidase. *Circ Res* 89: 47-54, 2001.
26. Gracey, AY, Troll, JV, Somero, GN. Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proc Natl Acad Sci U S A* 98: 1993-1998, 2001.
27. Green, H, MacDougall, J, Tarnopolsky, M, Melissa, NL. Downregulation of Na<sup>+</sup>-K<sup>+</sup>-ATPase pumps in skeletal muscle with training in normobaric hypoxia. *J Appl Physiol* 86: 1745-1748, 1999.
28. Hellwig-Burgel, T, Rutkowski, K, Metzen, E, Fandrey, J, Jelkmann, W. Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. *Blood* 94: 1561-1567, 1999.
29. Holloszy, JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 242: 2278-2282, 1967.
30. Hoppeler, H, Fluck, M. Plasticity of skeletal muscle mitochondria: structure and function. *Med Sci Sports Exerc* 35: 95-104, 2003.
31. Hoppeler, H, Kleinert, E, Schlegel, C, Claassen, H, Howald, H, Kayar, SR, Cerretelli, P. Morphological adaptations of human skeletal muscle to chronic hypoxia. *Int J Sports Med* 11 Suppl 1: S3-9, 1990.
32. Howald, H, Pette, D, Simoneau, JA, Uber, A, Hoppeler, H, Cerretelli, P. Effect of chronic hypoxia on muscle enzyme activities. *Int J Sports Med* 11 Suppl 1: S10-14, 1990.
33. Hudson, MB, Hosick, PA, McCaulley, GO, Schrieber, L, Wrieden, J, McAnulty, SR, Triplett, NT, McBride, JM, Quindry, JC. The effect of resistance exercise on humoral markers of oxidative stress. *Med Sci Sports Exerc* 40: 542-548, 2008.
34. Jessup, JV, Horne, C, Yarandi, H, Quindry, J. The effects of endurance exercise and vitamin E on oxidative stress in the elderly. *Biol Res Nurs* 5: 47-55, 2003.
35. Jewell, UR, Kvietikova, I, Scheid, A, Bauer, C, Wenger, RH, Gassmann, M. Induction of HIF-1alpha in response to hypoxia is instantaneous. *FASEB J* 15: 1312-1314, 2001.
36. Jezek, P, Plecita-Hlavata, L. Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia. *Int J Biochem Cell Biol* 41: 1790-1804, 2009.

37. Kayser, B, Hoppeler, H, Claassen, H, Cerretelli, P. Muscle structure and performance capacity of Himalayan Sherpas. *J Appl Physiol* 70: 1938-1942, 1991.
38. Koves, TR, Li, P, An, J, Akimoto, T, Slentz, D, Ilkayeva, O, Dohm, GL, Yan, Z, Newgard, CB, Muoio, DM. Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J Biol Chem* 280: 33588-33598, 2005.
39. Laughner, E, Taghavi, P, Chiles, K, Mahon, PC, Semenza, GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 21: 3995-4004, 2001.
40. Lin, J, Wu, H, Tarr, PT, Zhang, CY, Wu, Z, Boss, O, Michael, LF, Puigserver, P, Isotani, E, Olson, EN, Lowell, BB, Bassel-Duby, R, Spiegelman, BM. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 418: 797-801, 2002.
41. Lundby, C, Gassmann, M, Pilegaard, H. Regular endurance training reduces the exercise induced HIF-1alpha and HIF-2alpha mRNA expression in human skeletal muscle in normoxic conditions. *Eur J Appl Physiol* 96: 363-369, 2006.
42. MacDougall, JD, Green, HJ, Sutton, JR, Coates, G, Cymerman, A, Young, P, Houston, CS. Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. *Acta Physiol Scand* 142: 421-427, 1991.
43. Martin, D, O'Kroy, J. Effects of acute hypoxia on the VO2 max of trained and untrained subjects. *J Sports Sci* 11: 37-42, 1993.
44. Martinelli, M, Winterhalder, R, Cerretelli, P, Howald, H, Hoppeler, H. Muscle lipofuscin content and satellite cell volume is increased after high altitude exposure in humans. *Experientia* 46: 672-676, 1990.
45. McAnulty, SR, Hosick, PA, McAnulty, LS, Quindry, JC, Still, L, Hudson, MB, Dibarnardi, AN, Milne, GL, Morrow, JD, Austin, MD. Effect of pharmacological lowering of plasma urate on exercise-induced oxidative stress. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 32: 1148-1155, 2007.
46. Meeuwsen, T, Hendriksen, IJ, Holewijn, M. Training-induced increases in sea-level performance are enhanced by acute intermittent hypobaric hypoxia. *Eur J Appl Physiol* 84: 283-290, 2001.
47. Melissa, L, MacDougall, JD, Tarnopolsky, MA, Cipriano, N, Green, HJ. Skeletal muscle adaptations to training under normobaric hypoxic versus normoxic conditions. *Med Sci Sports Exerc* 29: 238-243, 1997.

48. Mingrone, G, Manco, M, Calvani, M, Castagneto, M, Naon, D, Zorzano, A. Could the low level of expression of the gene encoding skeletal muscle mitofusin-2 account for the metabolic inflexibility of obesity? *Diabetologia* 48: 2108-2114, 2005.
49. Mollard, P, Woorons, X, Letournel, M, Lamberto, C, Favret, F, Pichon, A, Beaudry, M, Richalet, JP. Determinants of maximal oxygen uptake in moderate acute hypoxia in endurance athletes. *Eur J Appl Physiol* 100: 663-673, 2007.
50. Norrbom, J, Sundberg, CJ, Ameln, H, Kraus, WE, Jansson, E, Gustafsson, T. PGC-1alpha mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J Appl Physiol* 96: 189-194, 2004.
51. Nourooz-Zadeh, J, Tajaddini-Sarmadi, J, Wolff, SP. Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Anal Biochem* 220: 403-409., 1994.
52. Pages, G, Milanini, J, Richard, DE, Berra, E, Gothie, E, Vinals, F, Pouyssegur, J. Signaling angiogenesis via p42/p44 MAP kinase cascade. *Ann N Y Acad Sci* 902: 187-200, 2000.
53. Peltonen, JE, Tikkanen, HO, Rusko, HK. Cardiorespiratory responses to exercise in acute hypoxia, hyperoxia and normoxia. *Eur J Appl Physiol* 85: 82-88, 2001.
54. Quindry, JC, McAnulty, SR, Hudson, MB, Hosick, P, Dumke, C, McAnulty, LS, Henson, D, Morrow, JD, Nieman, D. Oral quercetin supplementation and blood oxidative capacity in response to ultramarathon competition. *Int J Sport Nutr Exerc Metab* 18: 601-616, 2008.
55. Quindry, JC, Stone, WL, King, J, Broeder, CE. The effects of acute exercise on neutrophils and plasma oxidative stress. *Med Sci Sports Exerc* 35: 1139-1145, 2003.
56. Reynafarje, B. Myoglobin content and enzymatic activity of human skeletal muscle--their relation with the process of adaptation to high altitude. *Tech Doc Rep SAMTDR USAF Sch Aerosp Med SAM-TDR-62-89*: 8p, 1962.
57. Richard, DE, Berra, E, Pouyssegur, J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. *J Biol Chem* 275: 26765-26771, 2000.
58. Robergs, RA, Quintana, R, Parker, DL, Frankel, CC. Multiple variables explain the variability in the decrement in VO2max during acute hypobaric hypoxia. *Med Sci Sports Exerc* 30: 869-879, 1998.
59. Semenza, GL. Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* 15: 551-578, 1999.
60. Siri, WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 9: 480-491; discussion 480, 492, 1993.

61. Squires, RW, Buskirk, ER. Aerobic capacity during acute exposure to simulated altitude, 914 to 2286 meters. *Med Sci Sports Exerc* 14: 36-40, 1982.
62. Stroka, DM, Burkhardt, T, Desbaillets, I, Wenger, RH, Neil, DA, Bauer, C, Gassmann, M, Candinas, D. HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *FASEB J* 15: 2445-2453, 2001.
63. Terrados, N, Melichna, J, Sylven, C, Jansson, E, Kaijser, L. Effects of training at simulated altitude on performance and muscle metabolic capacity in competitive road cyclists. *Eur J Appl Physiol Occup Physiol* 57: 203-209, 1988.
64. Vogt, M, Puntchart, A, Geiser, J, Zuleger, C, Billeter, R, Hoppeler, H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. *J Appl Physiol* 91: 173-182, 2001.
65. Wayner, DDM, Burton, GW, Ingold, KU, Barclay, LRC, Locke, SJ. The relative contribution of vitamin E, urate, ascorbate and proteins to the total paroxyl radical-trapping antioxidant activity of human blood plasma. *Biochimica et biophysica acta* 924: 408-419, 1987.
66. Wende, AR, Schaeffer, PJ, Parker, GJ, Zechner, C, Han, DH, Chen, MM, Hancock, CR, Lehman, JJ, Huss, JM, McClain, DA, Holloszy, JO, Kelly, DP. A role for the transcriptional coactivator PGC-1alpha in muscle refueling. *J Biol Chem* 282: 36642-36651, 2007.
67. Woorons, X, Mollard, P, Lamberto, C, Letournel, M, Richalet, JP. Effect of acute hypoxia on maximal exercise in trained and sedentary women. *Med Sci Sports Exerc* 37: 147-154, 2005.
68. Zhong, H, Chiles, K, Feldser, D, Laughner, E, Hanrahan, C, Georgescu, MM, Simons, JW, Semenza, GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 60: 1541-1545, 2000.
69. Zoll, J, Ponsot, E, Dufour, S, Doutreleau, S, Ventura-Clapier, R, Vogt, M, Hoppeler, H, Richard, R, Fluck, M. Exercise training in normobaric hypoxia in endurance runners. III. Muscular adjustments of selected gene transcripts. *J Appl Physiol* 100: 1258-1266, 2006.

## **APPENDICES:**

### **Appendix 1: UM IRB Approval**



THE UNIVERSITY OF MONTANA-MISSOULA  
Institutional Review Board (IRB)  
for the Use of Human Subjects in Research  
CHECKLIST / APPLICATION

IRB Protocol No.:

156-11

At The University of Montana (UM), the Institutional Review Board (IRB) is the institutional review body responsible for oversight of all research activities involving human subjects outlined in the U.S. Department of Health and Human Services Office of Human Research Protection ([www.hhs.gov/ohrp](http://www.hhs.gov/ohrp)) and the National Institutes of Health, Inclusion of Children Policy Implementation (<http://grants.nih.gov/grants/funding/children/children.htm>).

**Instructions:** A separate registration form must be submitted for each project. IRB proposals are approved for three years and must be continued annually. Faculty and students may email the completed form as a Word document to [IRB@umontana.edu](mailto:IRB@umontana.edu) or submit a hardcopy to the Office of the Vice President for Research & Development, University Hall 116. Student applications must be accompanied by email authorization by the supervising faculty member or a signed hard copy.

All fields must be completed. If an item does not apply to this project, write in: n/a.

**1. Administrative Information**

Project Title: The impact of hypoxia and carbohydrate feedings on recovery from exercise	
Principal Investigator: Brent C. Ruby, PhD	Title: Professor
Email address: <a href="mailto:brent.ruby@mso.umt.edu">brent.ruby@mso.umt.edu</a>	
Work Phone: 406-243-2117	Cell Phone: 406-396-4382
Department: HHP	Office location: McGill 244

**2. Human Subjects Protection Training** (All researchers, including faculty supervisors for student projects, must have completed a self-study course on protection of human research subjects **within the last three years** (<http://www.umt.edu/research/complianceinfo/IRB/>) and be able to supply the "Certificate(s) of Completion" upon request. Add rows to table if needed.)

NAME and DEPT.	PI	CO-PI	Faculty Supervisor	Research Assistant	DATE COMPLETED Human Subjects Protection Course
Brent C. Ruby	X	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	3/2009
Charles Dumke	<input type="checkbox"/>	X	<input type="checkbox"/>	<input type="checkbox"/>	10/2008
John Cuddy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	X	3/2009
Walter Hailes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	X	2/2009

**3. Project Funding** (If federally funded, you must submit a copy of the abstract.)

Is grant application currently under review at a grant funding agency? <input type="checkbox"/> Yes (If yes, cite sponsor on ICF if applicable) <input checked="" type="checkbox"/> No		Has grant proposal received approval and funding? <input checked="" type="checkbox"/> Yes (If yes, cite sponsor on ICF if applicable) <input type="checkbox"/> No		
Agency	Grant No.	Start Date	End Date	PI
Defense Medical Research and Development Program	W81XWH-10-2--0120	10/1/2010	9/30/2013	Brent Ruby
Is this part of a thesis or dissertation? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No If yes, whose? _____		If yes, date you successfully presented your proposal to your committee: n/a		

**IRB Determination:**

For UM-IRB Use Only

- ☐ Approved Exempt from Review, Exemption # \_\_\_\_\_ (see memo)  
☐ Approved by Expedited Review, Category # \_\_\_\_\_ (see \*Note to PI)  
☐ Full IRB Determination  
☐ Approved (see \*Note to PI)  
☐ Conditional Approval (see memo) - IRB Chair Signature/Date: \_\_\_\_\_  
☐ Conditions Met (see \*Note to PI)  
☐ Resubmit Proposal (see memo)  
☐ Disapproved (see memo)

**\* Note to PI:** Study is approved for one year. Use any attached IRB-approved forms (signed/dated) as "masters" when preparing copies. If continuing beyond the expiration date, a continuation report must be submitted. Notify the IRB if any significant changes or unanticipated events occur. Notify the IRB in writing when the study is terminated

Risk Level: \_\_\_\_\_

Final Approval by IRB Chair: \_\_\_\_\_

Date: 8-29-2011 Expires: 8-28-2012

162





Montana Center for Work Physiology and Exercise Metabolism

## SUBJECT INFORMATION AND CONSENT FORM

**PROJECT IN BRIEF:** The impact of hypoxia and carbohydrate feedings on recovery from exercise

**SPONSOR:** Defense Medical Research and Development Program, a Department of Defense (DOD) Organization

**RESEARCHERS:** Dr. Brent Ruby, PhD (406) 243-2117  
Dr. Charles Dumke, PhD  
John Cuddy  
Walter Hailes

The University of Montana  
Montana Center for Work Physiology and Exercise Metabolism  
32 Campus Drive  
McGill Hall – HHP  
Missoula, MT 59812  
(406) 243 – 2117 (Dr. Brent Ruby, PhD)

*Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.*

### REQUIREMENTS

This research study requires that you meet the following criteria:

- Participants must be males between the ages of 18 and 40.

### PURPOSE OF THE STUDY

The study is designed to address the issue in current hypoxia research of recovery after exercise. This study will address how recovering in a hypoxic environment may affect muscle glycogen, specific mitochondrial genes related to exercise training, and oxidative stress.

### TEST PROCEDURES

3 VISITS TO THE LABORATORY WILL BE REQUIRED (18 HOURS), AS SUMMARIZED BELOW

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8/29/2012  
Date Approved By UM-IRB 8/29/2011  
IRB-Chair

### PRE TESTING (Visit 1)

1. A pre-screening assessment which involves a health/exercise questionnaire (Par-Q) and question regarding prior acute mountain sickness.
  - a. Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease.
  - b. Prior to any testing, you will be excluded from the study if you have previously had serious acute mountain sickness.
2. If you successfully complete the PAR-Q, you will then provide written informed consent following the reading of this document.
3. A measure of percent body fat obtained using underwater weighing
  - a. This test session will require that you do not eat for a minimum of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighing tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 20 minutes.
4. A maximal cycle ergometer test to measure aerobic fitness
  - a. This test will consist of cycling on a laboratory treadmill to volitional fatigue. The workload of the cycle ergometer will increase every three minutes and will progress to fatigue. You will be encouraged to continue until volitional fatigue, the point at which you can no longer continue cycling. During this test you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise so we can determine the appropriate exercise intensities for your experimental trial rides. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 30 minutes. You will be asked to fast for approximately 3 hours prior to this test.

### EXPERIMENTAL TRIALS (Visits 2 and 3)

**Trial 1)** cycling for 90 minutes at varying intensities in laboratory at 975 meters (Missoula, MT elevation) followed by 6 hour recovery in environmental chamber at 975 meters altitude

**Trial 2)** cycling for 90 minutes at varying intensities in laboratory at 975 meters (Missoula, MT elevation) followed by 6 hour recovery in environmental chamber at a simulated 5000 meters altitude

*\*m = meters above sea level*

#### *Experimental Protocol*

Following a controlled diet (with NO alcohol consumption) and exercise plan the day before and after an overnight fast, you will arrive to the laboratory in the early morning following a 12 hour

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB 8-29-2011  
[Signature] IRB-Chair

fast. A blood sample will be taken from an arm vein in your forearm and a muscle biopsy will be taken from the *vastus lateralis* (quad muscle) before commencement of exercise (Pre). Trials will be completed in a randomized order, but you will complete each of the two trials (7-14 days in between trials). The exercise protocol will be completed on a cycle ergometer in the laboratory. You will complete a 10-minute warm up at approximately 55% peak  $\text{VO}_2$ . Thereafter, you will complete a series of ten intervals, which includes two minutes at approximately 80% peak  $\text{VO}_2$  followed by four minutes at approximately 50% peak  $\text{VO}_2$ . After the series of 10 intervals, you will complete 8 minutes at 60% peak  $\text{VO}_2$  followed by 12 minutes at 50% peak  $\text{VO}_2$ . Total cycle time will be 90 minutes. You will be provided ~600 mL of water during the ride. Immediately upon cessation of exercise, a blood sample will be drawn and muscle biopsy will be taken from a separate incision ~2 cm above the Pre muscle biopsy (Post). You will then void if necessary, have body weight measured, and change clothing into a standardized shorts and t-shirt. You will then recover for 6 hours (sitting or lying) in the environmental chamber [22°C (72°F), 40% relative humidity] (in a random selection, one day will be hypoxic 5000 meters, and the other normoxic 975 meters). During this 6 hour recovery you will be supervised at all times by research staff. You will be provided a carbohydrate beverage at 0 hours into the recovery, and a solid food feeding at 2 and 4 hours into the recovery. Each feeding will be at a carbohydrate amount of 1.2 g/kg of body weight. During the recovery, blood samples will be taken at 2, 4, and 6 hours; metabolic gas collections will be taken 30 minutes into the recovery, and at 2, 4, and 6 hours into the recovery. Water will be provided ad-libitum during the 6 hour recovery. Following the 6 hour recovery, you will have a muscle biopsy taken from a separate incision ~2 cm above the Post muscle biopsy and an additional blood sample.

#### *Biopsies*

A total of 6 (3 per trial x 2 trials) muscle biopsies (3 from each leg) will be obtained from the front of your thigh muscle (*vastus lateralis*, approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the site be sterilized. After the site is cleaned, a small amount of local anesthesia (lidocaine) will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the site on the leg. After the area is treated with the lidocaine (approximately 5 mL, 1% lidocaine), a small incision (approximately 1/4 inch long) will be made through the skin and the outer covering (fascia) of your muscle to a depth of approximately 3/4-1.5 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips and/or a single stitch and bandaid and wrapped with a compression bandage. The biopsy samples will be obtained a) before the exercise session, b) after the exercise session, and c) following the 6 hours of recovery (biopsies for each trial will be on the same leg, above the initial or previous sample). This will be repeated for the second trial using the opposite leg. The muscle biopsies will be used to evaluate alterations in specific mitochondrial genes and muscle carbohydrate utilization kinetics in response to physical activity. Latex free bandages will be provided if subjects have a known allergy to latex. All of the muscle biopsies will be conducted by Dr. Brent Ruby or Dr. Charles Dumke.

#### *Blood Samples*

A total of 10 blood samples (5 per trial) will be collected using a venipuncture technique. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. These

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB-8-29-2011  
IRB-Chair



samples will be collected to measure blood glucose and insulin. All of the blood samples will be obtained under the direction of Dr. Brent Ruby or Dr. Charles Dumke. Blood samples will be taken before exercise, and then at intervals 0, 2, 4, and 6 hours into the recovery. ~10 mL will be drawn each time for a total of ~50 mL per trial. Blood samples will be used to evaluate alterations in oxidative stress biomarkers.

#### *Carbohydrate Beverage/Solid Feedings*

Immediately following exercise, you will be provided a standardized high-carbohydrate drink. You will be asked to consume this drink as quickly as possible without upsetting your stomach. The amount of carbohydrate consumed at each feeding time point (Post ride, 2 and 4 hours in recovery) will be 1.2 g/kg.

#### *Metabolic Gas Measurements*

Expiratory gasses will be measured during the 6 hour recovery at 30 min, 2, 4, and 6 hours. This requires you to breathe through a mouthpiece while wearing a nose clip, the same setup that will be used during the maximal oxygen uptake test.

#### *Dietary and Activity Recall*

For 24-hours before your first exercise trial you will be asked to record the foods and quantity that you consume. You are not allowed to consume any alcohol during this time period. For the second trial, you will consume the same foods and quantity of those foods that you consumed for the first trial. 2 days before your first trial day you can exercise as you wish, but this must be repeated at the same time of day and the same exercise prior to the second trial. For the 24-hours before each trial you cannot participate in any physical exercise.

#### *Body Weight*

Nude body weight will be measured in private on a calibrated scale. Weights will be taken before, during recovery, and after each trial.

#### *Urine*

You will be asked to void your bladder before each trial. After the initial void, urine will be collected in a disposable plastic container and urine volume will be measured for the duration of each trial.

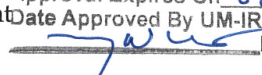
#### *Pulse Oximetry*

Spot check measurements throughout the exercise trial and 6 hour recovery will be taken using a pulse oximeter to check for blood oxygen saturation. The measurements will be taken pre exercise, after cycling for 45 minutes, post exercise, and every hour during the 6 hour recovery.

### **RISKS AND DISCOMFORTS**

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB 8-29-2011  
 IRB-Chair

pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. The laboratory has standard emergency procedures should any potential problems arise.

4. Symptoms of dehydration such as headache and general fatigue may result during and after the exercise.
5. You will be informed of any new findings that may affect your decision to remain in the study.
6. The muscle biopsy and blood sampling techniques may cause some local and temporary discomfort. It is normal to have the sensation of a deep tissue bruise around the site of the muscle biopsy. This pain should be manageable and not above the pain associated from a "charlie horse" type bruise. Risks involved with muscle biopsies include: nerve damage, moderate stiffness, hematoma, minimal scarring, bleeding, fainting, and seizure.
7. There is a minor risk of infection associated with blood sampling and the muscle biopsy. Should you notice unusual redness, swelling or drainage at the biopsy incision site or at the sites of the blood sampling sites you should seek medical attention and then notify Brent Ruby, study director.
8. There are minimal risks associated with the use of lidocaine (the local anesthetic). Risks include: pain at the injection site, dizziness, confusion, shakiness, visual changes, nausea, and unusually slow heart rate. The risk of a reaction to the lidocaine is extremely low (approximately 1/1,000,000). However to minimize this risk, no more than 9 mL of a 1% lidocaine solution will be used per biopsy. You will be excluded from participation if you have a known history of allergic reactions to local anesthetics.
9. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.
10. During recovery in the 5000 meter simulated laboratory trial, you may experience acute mountain sickness and may experience the following symptoms: headache, fatigue, dyspnea, hyperventilation, gastrointestinal distress, and decreased thirst.

#### **PAYMENT FOR PARTICIPATION**

Payment will be according to the following scale:

Preliminary tests: \$50

Experimental trial #1: \$250

Experimental trial #2: \$250

Therefore, upon completion of the entire study, you will be paid a total of \$550. If you decide to withdraw at any time, you will be compensated for the test sessions you have completed.

#### **BENEFITS OF PARTICIPATION**

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB 8-29-2011  
 IRB-Chair

1. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.
2. There is no promise that you will receive any benefit as a result of taking part in this study.
3. The scientific benefit includes elucidating the effects of hypoxia and carbohydrate feedings on recovery on muscle glycogen, specific mitochondrial genes related to exercise training, and oxidative stress.

### **CONFIDENTIALITY**

1. Your records will be kept private and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files; representatives of the U. S. Army Medical Research and Materiel Command (or the DOD) are authorized to review research records.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data.

### **COMPENSATION FOR INJURY**

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. *In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University's Claim representative or University Legal Counsel.*

### **VOLUNTARY PARTICIPATION AND WITHDRAWAL**

It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive full compensation for all the test sessions you complete or initiate. A copy of this consent form will be provided for you at your request. In addition, the data collected during this study will be done at no cost to you.

### **QUESTIONS**

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Dr. Brent C. Ruby, PhD at

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB 8-29-2011  
 IRB-Chair



(406) 243-2117 (office) or (406) 396-4382. If you have any questions regarding your rights as a subject, you may contact the chair of the IRB through the University of Montana Research Office at (406) 243-6670.

#### STATEMENT OF CONSENT

I have read the above statements and understand the risks involved with this study. I authorize Dr. Brent C. Ruby, PhD and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Dr. Brent C. Ruby, PhD at home (406) 542-2513, cell (406) 396-4382 or at the Human Performance Laboratory (406) 243-2117.

Participant (print) \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_

#### Disclosure of Personal Health Information

My individual health information that may be used to conduct this research includes:

*Age, height, weight, %body fat, VO<sub>2</sub> max, gene expression in response to exercise/hypoxia, muscle glycogen levels, and markers of oxidative stress.*

I authorize *Dr. Brent C. Ruby, PhD* and the researcher's staff *and* representatives of the USAMRMC to use my individual health information for the purpose of conducting the research project entitled "The impact of hypoxia and carbohydrate feedings on recovery from exercise."

Since I receive compensation for participating in this study, identifying information about me may be used as necessary to provide compensation.

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

#### STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names and physiological data will not be associated with them.

Signature \_\_\_\_\_ Date \_\_\_\_\_

Project funded by the Defense Medical Research and Development Program, a Department of Defense (DOD) organization

Approval Expires On 8-28-2012  
Date Approved By UM-IRB 8-24-2011  
\_\_\_\_\_  
IRB-Chair

## **Appendix 2: US Army HRPO Approval (Email notification received 9/22/2011)**

Classification: UNCLASSIFIED

Caveats: NONE

SUBJECT: Initial Approval for Protocol, "The Impact of Hypoxia and Carbohydrate Feedings on Recovery from Exercise," Submitted by Brent C. Ruby, PhD, University of Montana, Missoula, Montana, in Support of the Proposal, "Evaluation of the Human/Extreme Environment Interaction: Implications for Enhancing Operational Performance and Recovery," Submitted by Brent C. Ruby, PhD, University of Montana, Missoula, Montana, Proposal Log Number DM090529, Award Number W81XWH-10-2-0120, HRPO Log Number A-16417.2

1. The subject protocol was approved by the University of Montana Institutional Review Board (IRB) on 29 August 2011. This protocol was reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protections Office (HRPO) and found to comply with applicable DOD, U.S. Army, and USAMRMC human subjects protection requirements.
2. This greater than minimal risk study is approved for the enrollment of 12 subjects.
3. Please note the following reporting obligations. Failure to comply could result in suspension of funding.
  - a. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protections Office (HRPO) for approval prior to implementation. Major modifications include changes in study design, a change in Principal Investigator, change or addition of an institution, change in age range, change in/addition to the study population or a change that could potentially increase risks to subjects. All other amendments must be submitted with the continuing review report to the HRPO for acceptance.
  - b. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and related a subject deaths must be promptly reported by phone (301-619-2165), by email (hrpo@amedd.army.mil), or by facsimile (301-619-7803) to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 504 Scott Street, Fort Detrick, Maryland 21702-5012.
  - c. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.
  - d. Any deviation to the protocol that may have an adverse effect on the safety or rights of the subject or the integrity of the study must be reported to the HRPO as soon as the deviation is identified.
  - e. A copy of the continuing review report and the re-approval notification by the University of Montana IRB must be submitted to the HRPO as soon as possible after receipt of approval. According to our records, it appears the current approval by the University of Montana IRB expires on 28 August 2012. Please note that the HRPO also conducts random audits at the time of continuing review and additional information and documentation may be requested at that time.
  - f. The final study report submitted to the University of Montana IRB, including a copy of any acknowledgement documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.
  - g. The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency



concerning this research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements must be reported immediately to the HRPO.

4. Please Note: The U S Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protections Office conducts random site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

5. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

6. The HRPO point of contact for this study is Sharon A. Evans, PhD, CIP, Human Subjects Protection Scientist, at 301-619-2256/sharon.a.evans.ctr@us.army.mil.

LAURA RUSE BROSCH, RN, PhD  
Director, Office of Research Protections  
Human Research Protection Office  
U.S. Army Medical Research and Materiel Command

Note: The official copy of this approval memo is housed with the protocol file at the Office of Research Protections, Human Research Protections Office, 504 Scott Street, Fort Detrick, MD 21702. Signed copies will be provided upon request.

Classification: UNCLASSIFIED

Caveats: NONE